to be mainly restricted by the plasma membrane permeability to anions (in isotonic salt and/or sugar media with 0.1 mM KCl, but not in KCl medium). Under these conditions, permeabilizing activity of the peptides was significantly higher in choline chloride and low ionic strength sugar media. Before swelling, fast shrinkage of the cells was caused by some relatively short peptides at concentrations of 0.25-2.0 μM in NaCl/sugar (1:1 osmotic contribution) or choline chloride media. The first derivative of the shrinkage traces coincided with the first derivative of the membrane potential drop. The rate of cell swelling decreased with an increase in the molecule size of sugar or polyethylene glycol. The initial phase of cell shrinkage was also observed in the absence of valinomycin, but at significantly higher peptide concentrations. We suggest that the cell shrinkage induced by polycationic peptides is due to anion selectivity of the peptide pores at the initial step of their formation. The obtained data contribute to a better understanding of the mechanism of biomembrane permeabilization by anticancer and antimicrobial polycationic peptides. (Colciencias grant #111840820380 and the National University of Colombia grant #20101007930).

1825-Pos Board B735
Three-State Discrete Kinetics of the OpdK Protein Pore
Belete R. Cheneke, Bert van den Berg, Liviu Movileanu.
OpdK protein is an 8 Å-wide diameter, kidney shaped pore located in the outer membrane of the Gram-negative bacteria. We reconstituted this protein into the planar lipid bilayers and measured the single-channel ionic current. We observed that the OpdK protein pore undergoes a three-state, discrete kinetics, whose current sub-states feature different probabilities of occupancy, rate constants and conductances. We have developed a simple kinetic model to account for the dynamics of spontaneous gating in the OpdK protein pore. Here, we discuss the nature of the gating dynamics of these three current sub-states, which is dependent on the concentration of the KCl in the chamber and applied transmembrane voltage. We propose that these well-defined current sub-states are due to fluctuations of short and long extracellular loops located near to the pore constriction.
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Assessing Pore Forming Capability of Protegrin-1 in Lipid Bilayers of Varying Cholesterol Content
Eddie Maldonado, Michael Henderson, Ka Yee Lee, Robert Lehrer, Alan J. Waring.
Pore formation affects the structural integrity of the lipid membrane, compromising its ability to act as a semi-permeable barrier and eventually leading to cell death. We present results obtained via atomic force microscopy (AFM) when lipid bilayer patches made dimyristoylphosphatidylcholine (DMPC) with increasing cholesterol content were subjected to the action of an cationic antimicrobial peptide (AMP), Protegrin-1 (PG-1). The cholesterol content was varied from 0% to 30%, where the lower and the upper bounds represent the cholesterol content in bacterial and mammalian cells, respectively. In the absence of cholesterol, PG-1 penetrated into the bilayer, leading to pore formation.

1827-Pos Board B737
Transmembrane Pores Formed by Human Antimicrobial Peptide LL-37
Chang-Chun Lee, Yen Sun, Chih-Wei Chen, Shuo Qian, Huey W. Huang.
Human LL-37 is a multifunctional cathelicidin peptide which has shown a wide spectrum of antimicrobial activity by causing membrane permeabilization similar to other antimicrobial peptides (AMPs), but its molecular mechanism has not been clarified. Two independent experiments found LL-37 bound to membranes in the alpha-helical form with the axis lying in the plane of membrane. This led to the conclusion that the membrane permeabilization by LL-37 is a detergent-like effect, rather than by the formation of stable pores, as exhibited by other well-studied AMPs, such as magainin. Here we report the discovery of stable transmembrane pores induced by LL-37. The formation of pores coincided with the detection of LL-37 helices aligning normal to the membrane. The new results were obtained by the realization that in the previous studies the rotation of LL-37 helices might be hindered in the multilayers. LL-37 helices are longer than the membrane thickness. In order for the membrane-bound LL-37 to freely rotate, the spacing between membranes must exceed the length of the peptide. This was achieved by swelling the lipid-peptide multilayers with excessive water to a swollen state. The transmembrane pores were detected and investigated in the swollen states by oriented circular dichroism, neutron in-plane scattering and X-ray lamellar diffraction. The results are consistent with the effect of LL-37 on giant unilamellar vesicles. The pores have a water channel of radius 23-33 Å. The molecular mechanism of pore formation by LL-37 is the two-state model exhibited by magainin and other smaller AMPs. This finding increases the likelihood that there is a common mechanism for most of AMPs. Whether this is the case is relevant to the principle of peptide designs for therapeutic applications.

1828-Pos Board B738
Kinetics of Membrane Permeabilization by Human Antimicrobial Peptide LL-37
Chih-Wei Chen, Yen Sun, Chang-Chun Lee, Huey W. Huang.
The molecular mechanism of membrane permeabilization by antimicrobial peptides (AMPs) has been an active field of research. There are suggestions that different AMPs might act with different mechanisms. One example used to support this hypothesis is LL-37 which was found by two independent studies that the helical peptide adsorbs to the membrane with its helical axis parallel to the membrane. Base on this result, it was concluded that LL-37 does not form pores, unlike many well studied AMPs, such as magainin. We have recently found that this result was due to the impediment of peptide rotation in the multilayers, because LL-37 is longer than the membrane thickness. We have detected pores by LL-37 in swollen multilayers by neutron in-plane scattering and also found that the helices were oriented normal to the membrane, exactly like magainin. We would like to know if the pore formation by long helices may exhibit kinetic potential barriers. By observing individual giant unilamellar vesicles (GUVs), we found that the pores are stable and of finite size consistent with neutron measurement. However, sometimes the pores reduce their sizes in time, allowing only partial leakage of large dye molecules, while small dye molecules completely leaked out. Both the formation of the pores and change of pore size occur stochastically as if there are potential barriers for each type of event. We also used the method of aspiration to correlate the leakage with the change of aspiration protrusion length. The maximum protrusion length before pore formation depends on the peptide concentration, exhibiting yet another type of potential barrier.

1829-Pos Board B739
Protective Bacterial Toxin TisB Produces Well-Defined Anion-Selective Pores in Planar Lipid Bilayers
Philip A. Gurnev, Ron Ortenberg, Kim Lewis, Sergey M. Bezrukov.
Recently identified small bacterial peptide TisB is a component of a toxin/antitoxin system. TisB toxin induces formation of drug-tolerant persister cells in response to DNA damage. We have found that TisB forms well-defined ion-conductive pores in planar lipid bilayers. Using high-resolution conductance recordings with membranes of varying lipid compositions, we show that bath solution concentrations of TisB higher than 10 μM induce multilevel conductive states, which resemble pore formation by the well-known channel-former antibiotic alamethicin. In 1 M KCl TisB-induced pores usually first appear as stable conductive ohmic states (0.5, 1.5, and 2.6 nS), and, as time progresses, tend to produce various higher conductive states. The transition to these states is also favored by application of higher positive or negative transmembrane voltages. Both low and high conductive states possess close anion selectivity (~80 % anion current, measured in 1M/0.1M KCl salt gradient). Probing TisB pores in their lowest conductive states with differently-sized polyethylene glycols (PEGs) shows only minute polymer partitioning even for the smallest PEGs of 200 and 300 molecular weight. This finding implies that the lowest conductive states are characterized by relatively small diameter of the aqueous pores. TisB apparently creates a dormant state in persister cells by decreasing the protein motive force across their membranes and reducing ATP production, which leads to antibiotic tolerance.

1830-Pos Board B740
Interaction of Alpha-1 antitrypsin with Model Membranes
Philip A. Gurnev, Pat DeMoss, Kelly Schweitzer, Irina Petrache, Horia I. Petrache.
Alpha-1 antitrypsin (A1AT) is an abundant protease inhibitor, belonging to serpin superfamily. A1AT was found to penetrate the membranes by a mechanism that is not yet understood. In this study, we investigate the interaction of A1AT with planar lipid bilayers. We find that at bath solution concentrations higher than 10 μM, A1AT produces membrane instabilities and eventual rupture. The membrane-disrupting activity of A1AT correlates with the membrane content of the charged lipid, phosphatidylserine. Addition of